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Short Communication

Prenatal diagnosis and molecular cytogenetic characterization of a *de novo* 4.858-Mb microdeletion in 15q14 associated with *ACTC1* and *MEIS2* haploinsufficiency and tetralogy of Fallot

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ABSTRACT

Objective: To present prenatal diagnosis and molecular cytogenetic characterization of a *de novo* 15q14 microdeletion associated with tetralogy of Fallot (TOF).

Materials and methods: This was the first pregnancy of a 31-year-old primigravid woman. The pregnancy was uneventful until 23 weeks of gestation when TOF was first noted. The woman underwent amniocentesis at 23 weeks of gestation. Conventional cytogenetic analysis was performed using cultured amniocytes and parental bloods. Array comparative genomic hybridization (aCGH) was performed on uncultured amniocytes and parental bloods. Metaphase fluorescence *in situ* hybridization (FISH) was performed on cultured amniocytes. Quantitative fluorescent-polymerase chain reaction (QF-PCR) analysis was performed on placental tissue and parental bloods.

Results: Conventional cytogenetics on cultured amniocytes revealed a karyotype of 46,XY, aCGH on uncultured amniocytes revealed a *de novo* 4.858-Mb microdeletion in 15q14 encompassing *ACTC1* and *MEIS2*, and metaphase FISH analysis on cultured amniocytes confirmed a 15q14 microdeletion. Postnatal phenotype included facial dysmorphism. QF-PCR assays detected a paternal origin of the 15q14 microdeletion in the fetus.

Conclusion: Fetuses with 15q14 microdeletion may present TOF on the second-trimester ultrasound. aCGH and metaphase FISH are useful for rapid prenatal diagnosis of 15q14 microdeletion associated with TOF. A prenatal diagnosis of TOF should include a differential diagnosis of 15q14 microdeletion in addition to 22q11.2 deletion syndrome and other microdeletion syndromes.

Introduction

Clinical reports concerning patients with deletion including the cytogenetic band of 15q14 are very rare [1–17]. We previously reported a male patient with speech and language disorder, cleft palate, epilepsy, ventricular septal defect (VSD), mental retardation, developmental delay and facial dysmorphism of low-set ears,

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beaklike nose, prominent nasal bridge, narrow forehead, long face, pointed chin and dental position abnormalities, and a 5.6-Mb deletion in 15q14 (31,833,000–37,477,000 bp) [14]. Here, we additionally present prenatal diagnosis and molecular cytogenetic characterization of a *de novo* 4.858-Mb microdeletion in 15q14 (33,865,665–38,723,737 bp) associated with tetralogy of Fallot (TOF) and facial dysmorphism. To our knowledge, this is the first report of prenatal diagnosis of 15q14 microdeletion because of prenatal ultrasound abnormality of TOF. Our report emphasizes that prenatal diagnosis of TOF should include a differential diagnosis of 15q14 microdeletion in addition to 22q11.2 microdeletion syndrome and other microdeletion syndromes.

Materials and methods

Clinical description

This was the first pregnancy of a 31-year-old woman. Her husband was 50 years old, and there was no family history of congenital malformations. The pregnancy was uneventful until 23 weeks of gestation when TOF was first noted. The woman underwent amniocentesis at 23 weeks of gestation. Conventional cytogenetic analysis was performed using cultured amniocytes and parental bloods. Array comparative genomic hybridization (aCGH) was performed using uncultured amniocytes and parental bloods. No cytogenetic abnormality was found by conventional cytogenetics. However, aCGH and metaphase fluorescence *in situ* hybridization (FISH) detected a 15q14 microdeletion. The male fetus died at 26 weeks of gestation and postnatally manifested facial dysmorphism of low-set ears, beaklike nose, prominent nasal bridge, narrow forehead, long face, micrognathia and pointed chin, a birth length of 34.5 cm, and a birth weight of 992 g. Quantitative fluorescent polymerase chain reaction (QF-PCR) was performed using the DNA extracted from the placental tissue and the parental blood samples to determine the parental origin of the *de novo* deletion.

Conventional cytogenetic analysis

A routine cytogenetic analysis by G-banding techniques at the 550 bands of resolution was performed. Amniotic fluid and parental blood were collected, and the samples were subjected to cell culture according to the standard blood cytogenetic protocol.

aCGH

Whole-genome aCGH on the DNAs extracted from uncultured amniocytes and parental bloods was performed using CytoChip ISCA Array (Illumina, San Diego, CA, USA), which has 60,000 probes and a median resolution of 51 kb across the entire genome according to the manufacturer's instruction. The DNA from amniocytes was extracted through the manufacturer's protocol of a QIAamp DNA Mini kit (Qiagen, Inc., Valencia, CA, USA). Then, 0.65 μg of the extracted DNA was labeled with Cy3 dye whereas the same amount of normal female genomic DNA (gDNA, G1521; Promega, Madison, WI, USA) was labeled in Cy5 dye as a control. The experiment was performed according to the procedures recommended by the Illumina CytoChip Oligo Microarray Reference Guide. The data were presented using BlueFuse Multi software (Illumina, San Diego, CA, USA).

FISH

Metaphase FISH analysis was performed on cultured amniocytes using a 15q14-specific bacterial artificial chromosome (BAC) probe RP11-815C16 (34,182,800–34,368,355) [hg 19] (FITC, green spectrum) and a 15q12-specific BAC probe RP11-812O7 (25,928,899–26,127,069) [hg 19] (Texas red, red spectrum) according to the standard FISH protocol.

QF-PCR

QF-PCR analysis was performed on the DNA extracted from the placental tissue and the parental bloods. Briefly, primers specifically flanking short tandem repeat markers on chromosome 15 region such as D15S661 (15q11.1), D15S1232 (15q14), D15S194 (15q14), and D15S641 (15q15.1) were applied to undertake polymorphic marker analysis to determine the parental origin of genomic imbalance if detected.

Results

The G-banded chromosome analysis revealed a karyotype of 46,XY in cultured amniocytes and normal karyotypes in parental bloods. aCGH on the DNA extracted from the uncultured amniocytes detected a *de novo* 4.858-Mb microdeletion in 15q14 or arr 15q14 (33,865,665–38,723,737) × 1.0 (Figure 1). The deleted region contains 54 genes including 17 OMIM genes of *RYR3*, *AVEN*, *CHRM5*, *KATNBL1*, *EMC4*, *SLC12A6*, *NOP10*, *NUTM1*, *LPCAT4*, *GOLGA8A*, *GOLGA8B*, *GJD2*, *ACTC1*, *AQR*, *C15orf41*, *MEIS2*, and *SPRED1*. aCGH analysis of the parental blood samples did not reveal such a deletion. Metaphase FISH analysis showed the 15q14 microdeletion in 14/14 cultured amniocytes (Figure 2). QF-PCR assays detected a paternal origin of the 15q14 microdeletion in the fetus (Figure 3).

Discussion

The present case had a 4.858-Mb microdeletion in 15q14 encompassing the cardiac development candidate genes of *ACTC1* and *MEIS2*. Of interest is the association of 15q14 microdeletion and TOF on prenatal ultrasound in the present case. Congenital heart defects associated with the cases with the 15q14 microdeletion have been well described: Chen et al [14] reported VSD associated with del(15)(q14) (submicroscopic, 5.6 Mb); Crowley et al [15] reported VSD associated with del(15)(q14) (submicroscopic, 123 kb); Johansson et al [17] reported VSD in three of nine cases with del(15)(q14) (submicroscopic, 0.6–4.8 Mb); Brunetti-Pierri et al [13] reported congenital heart defects associated with del(15)(q14) (submicroscopic, 4.2 Mb) and del(15)(q13–q14) (submicroscopic, 8.9 Mb), respectively, in two patients; Erdogan et al [12] reported atrial septal defect (ASD) associated with del(15)(q14) (submicroscopic, 5.3 Mb); Galan et al [8] reported pulmonary valve stenosis associated with del(15)(q12–q14); Tonk et al [9] reported VSD, patent ductus arteriosus (PDA), and ischemic cardiomyopathy associated with del(15)(q12–q14); Autio et al [7] reported ASD associated with del(15)(q13–q15); Herva and Vuorinen [3] reported VSD, hypoplastic pulmonary artery, and atretic tricuspid valve associated with del(15)(q12–q14); Pauli et al [5] reported VSD associated with del(15)(pter-q15) and del(11)(q25-qter); Windpassinger et al [10] reported persistent foramen ovale and PDA associated with del(15)(pter-q14) and del(3)(qter); Duckett and Roberts [4] reported VSD, ASD, PDA, and transposition of great vessels associated with del(15)(pter-q14 or q15) and trisomy 13 (pter-q32 or q33); Schwartz et al [6] reported coarctation of the aorta and PDA associated with del(15)(pter-q14) and del(22)(pter-q13.2); and Matsumura et al [11] reported PDA associated with del(15)(pter-q14) and trisomy 22q.

ACTC1 or actin, α-cardiac muscle (OMIM 102540) is identified in muscle and has been suggested to be associated with congenital heart defects. Matsson et al [18] performed *ACTC1*

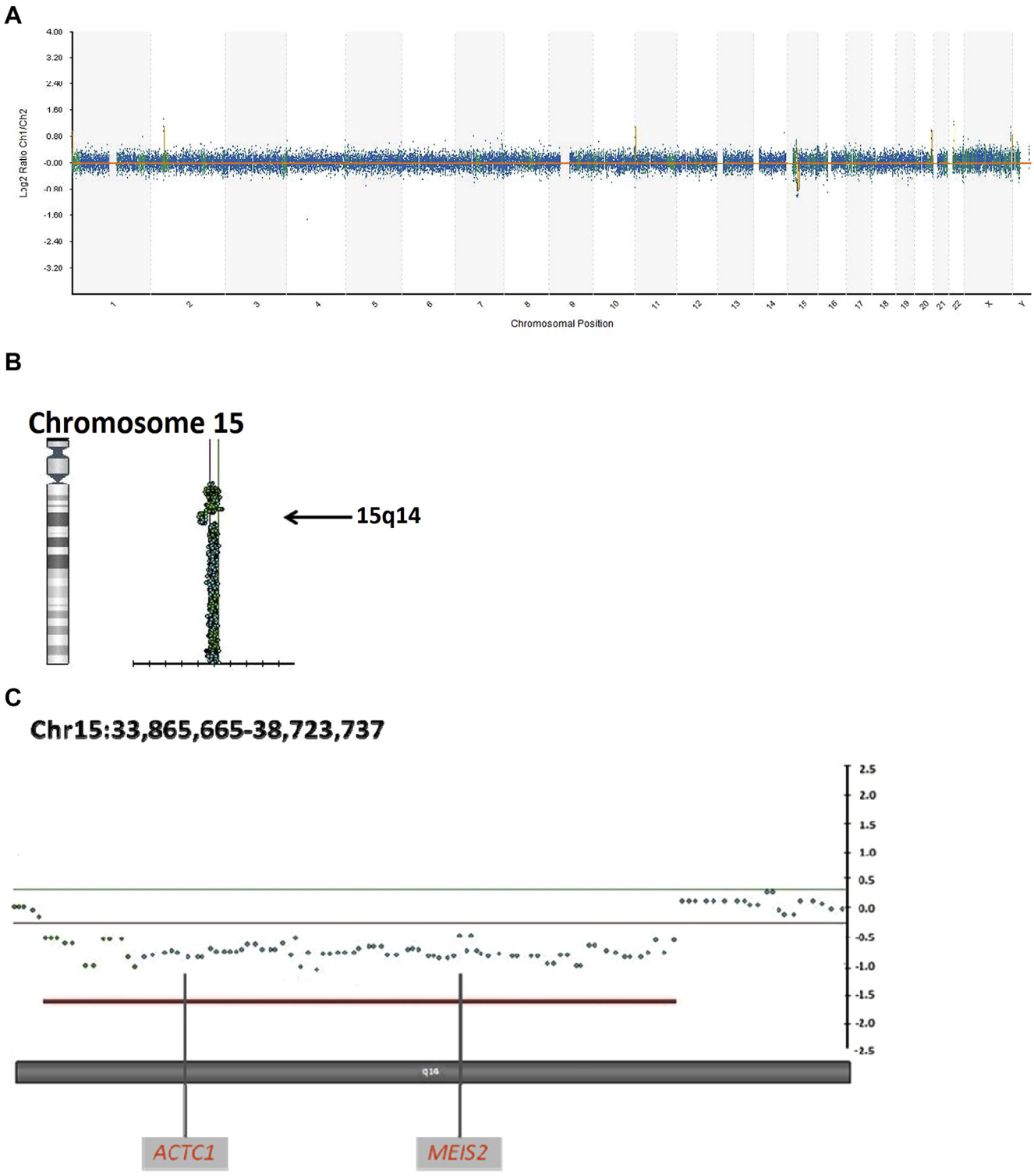

Figure 1. Array comparative genomic hybridization of the DNA extracted from the uncultured amniocytes shows a 4.858-Mb microdeletion in 15q14 or arr 15q14 (33,865,665–38,723,737) × 1.0. The deleted region contains the genes of *ACTC1* and *MEIS2*. (A) Whole genome view. (B and C) Zoom-in view.

knockdown in chick embryos, and found delayed and reduced atrial septa and suggested α-cardiac actin mutations produce ASDs. Heterozygous mutations in the *ACTC1* gene have been associated with dilated cardiomyopathy 1R (*CMD1R*) (OMIM 613424), hypertrophic cardiomyopathy 11 (*CMH11*) (OMIM 612098), left ventricular noncompaction 4 (*LVNC4*) (OMIM 613424), and atrial septal defect 5 (*ASD5*) (OMIM 612794). Matsson et al [18] analyzed two large Swedish families segregating autosomal dominant secundum *ASD5* and identified heterozygosity for a mutation of M123V in the 20 affected individuals. Matsson et al [18] also identified a 17-bp deletion in the *ACTC1* gene in a 10-year-old girl with secundum ASD.

MEIS2 or myeloid ectopic viral insertion site, mouse homolog of, 2 (OMIM 601740) is a homeodomain-containing transcription

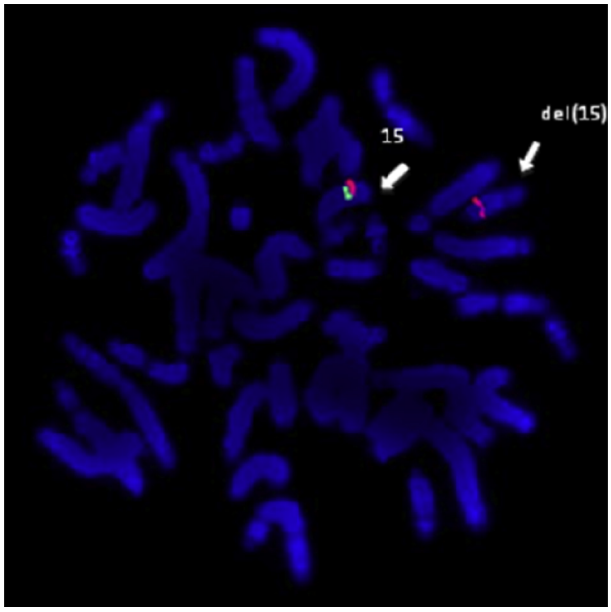

Figure 2. Metaphase fluorescence *in situ* hybridization on the cultured amniocytes using a 15q14-specific bacterial artificial chromosome (BAC) probe RP11-815C16 (34,182,800–34,368,355) [hg 19] (FITC, green spectrum) and a 15q12-specific BAC probe RP11-812O7 (25,928,899–26,127,069) [hg 19] (Texas red, red spectrum) shows both red and green signals in the normal chromosome 15 (chr15) and only the red signal in the aberrant chromosome 15 or del(15).

factor of the TALE superfamily and has been suggested to be associated with congenital heart defects and cleft palate [12,13,15–22]. Stankunas et al [19] demonstrated that in the mice model, disruption of *MEIS*, which encodes a Pbx DNA-binding partner, results in a full spectrum of cardiac anomalies involving the outflow tract resembling those caused by mutations of *Pbx* genes. Crowley et al [15] reported a male infant with cleft palate, VSD, bilateral hearing loss, and a mosaic deletion of a 123-kb region (34,978,029–35,100,886) encompassing only the *MEIS2*, and suggested that *MEIS2* is involved in the development of congenital heart defects and cleft palate. Paige et al [20] demonstrated that in the zebrafish model, *MEIS2* is critical for proper heart tube formation and subsequent cardiac looping. Johansson et al [17] suggested that in syndromic cases of congenital heart defects and orofacial clefting, *MEIS2* should be considered among the candidate genes in cases without 22q11.2 deletions. Louw et al [21] reported a *de novo* small intragenic mutation in *MEIS2* in a female patient with cleft palate, septal defects, aortic coarctation, facial dysmorphism, intellectual disability, and autism spectrum disorder, and suggested that *MEIS2* is involved in cardiac development, cleft palate, and intellectual disability. Machon et al [22] demonstrated that *MEIS2* plays a critical role during cranial and cardiac neural crest cell development in the mouse.

In the present case, the conventional cytogenetic analysis of cultured amniocytes revealed a karyotype of 46,XY, whereas aCGH analysis on uncultured amniocytes and metaphase FISH analysis on cultured amniocytes revealed a 15q14 microdeletion. The aCGH analysis on uncultured amniocytes has the advantage of detection of microdeletion syndromes in the fetuses with a normal karyotype by conventional cytogenetic analysis [23,24].

Patients with 22q11.2 deletion syndrome are associated with a high rate of congenital heart defects, especially conotruncal malformations, TOF, interrupted aortic arch, VSD, and truncus arteriosus, as well as a high rate of palatal malformations. However, various chromosomal loci and genes have been responsible for congenital heart defects and TOF in addition to *TBX1* (22q11.2; OMIM 602054) such as *CFC1* (2q21.1; OMIM 605194), *NKX2-5*

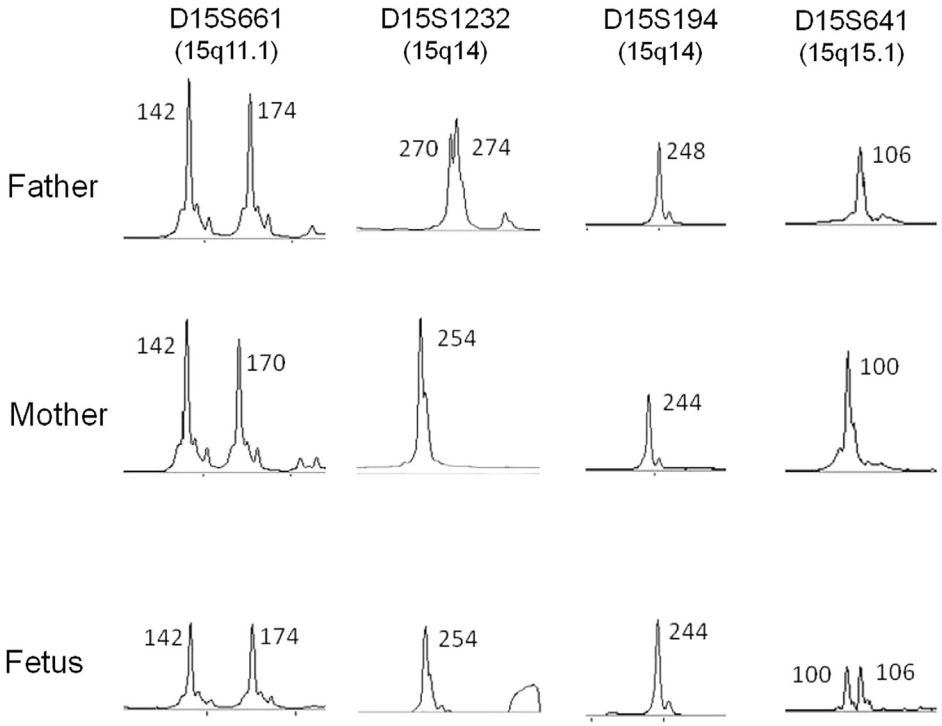

Figure 3. Representative electrophoretograms of quantitative fluorescent-polymerase chain reaction assays on the DNA extracted from placental tissue and parental bloods. The informative markers D15S1232 (15q14) and D15S194 (15q14) show only one peak of fluorescent activity from the maternal allele in the fetus, indicating a paternal origin of the 15q14 microdeletion in the fetus.

(5q35.1; OMIM 600584), *NKX2-6* (8p21.2; OMIM 611770), *ZFPM2* (8q23.1; OMIM 603693), *GATA6* (18q11.2; OMIM 601656), *GDF1* (19p13.11; OMIM 602880), and *JAG1* (20p12.2; OMIM 601920) [25]. The present case provides evidence that fetuses with 15q14 microdeletion may present TOF on the second-trimester ultrasound. We suggest that prenatal diagnosis of TOF should include a differential diagnosis of 15q14 microdeletion in addition to 22q11.2 microdeletion syndrome and other microdeletion syndromes.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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